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Novel Proteins Interacting with the Leucine-rich Repeat Domain of Human Flightless-I Identified by the Yeast Two-Hybrid System

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The *flightless-I* gene encodes a member of the gelsolin-like family of actin-binding proteins linked to a leucine-rich repeat (LRR) domain. It is required for cellularization during early embryogenesis and normal development of the indirect flight muscles in *Drosophila melanogaster*. Although the association between actin and the gelsolin-like domain of the human Flightless-I homologue (FLI) has been established, its biological role is unknown. The human *FLI* gene is mapped within the Smith–Magenis microdeletion region of chromosome 17. We report the identification of two related genes, *LRRFIP1* and *LRRFIP2*, encoding proteins that interact with the LRR domain of human FLI using the yeast two-hybrid system. LRRFIP1 exhibits sequence identity with the TRIP RNA-binding protein and GCF-2 transcriptional repressor, which are also related to the murine FLAP-1 gene. *LRRFIP2* is a novel gene that shares sequence homology with *LRRFIP1* and FLAP-1. *LRRFIP1* and *LRRFIP2* both express alternative splice variants in heart and skeletal muscle tissue. A coiled-coil domain, conserved within each encoded protein, serves as a potential interaction motif for FLI LRR. The occurrence of multiple proteins able to interact with FLI within the same tissue suggests that they may compete for the same binding site. Sequencing and PCR-directed genomic analysis indicate that *LRRFIP1* and *LRRFIP2* are related genes that arose from gene duplication.

INTRODUCTION

The *flightless-I* gene is required for embryogenesis and flight muscle function in *Drosophila melanogaster*. Homologues have been isolated from *Caenorhabditis elegans* and from humans (Campbell *et al.*, 1993). It encodes a modular protein consisting of a leucine-rich repeat (LRR) domain linked to a carboxyl-terminal sequence similar to gelsolin. Gelsolin-like proteins constitute a family of actin-binding proteins capable of either severing or nucleating filaments in a calcium-sensitive manner and regulated by PIP_2 (Yin *et al.*, 1988; Kwiatkowski *et al.*, 1989; reviewed in Weeds and MacIver, 1993). Interaction between actin and the gelsolin-like domain (GLD) from the human homologue of Flightless-I protein (FLI) has been demonstrated *in vitro* (Orloff *et al.*, 1995; Liu and Yin, 1998). Though the interaction between actin and *Drosophila* FLI GLD has not been demonstrated, a homologous function is assumed based on their sequence conservation especially within critical actin-binding regions (de Couet *et al.*, 1995). A biological basis for the association with actin, however, has been examined only by genetic analysis.

A missense mutation within the *Drosophila* FLI GLD leads to the severe disruption of myofibrillar organization in the indirect flight muscle cytoskeleton (Miklos and de Couet, 1990; de Couet *et al.*, 1995). Sarcomeres appear malformed and fragmented as actin filaments fray toward the periphery of the myofibril. In many instances, myofibrils are void of recognizable sarcomeres, leaving filament bundles to be strewn. These areas are also associated with the occurrence of dense, striated bundles that have the structural appearance of Z-band material (Miklos and de Couet, 1990). The disorganized myofibrillar arrangement suggests a defect during the development of indirect flight muscles. It is thus argued that FLI has a regulatory role in establishing myofibrillar organization rather than being a structural component holding the flight muscle cytoskeleton together.

In a more extreme case, null alleles fail to form a cellularized blastoderm during early embryogenesis. The process of cellular morphogenesis is dependent upon the coordination of actin cytoskeleton dynamics by associated proteins (reviewed in Schejter and Wieschaus, 1993; Miller and Kiehart, 1995). Elimination or alteration of the maternal supply of *flightless-I* gene product to the fertilized egg causes incomplete cellularization of the syncytial blastoderm, resulting in ab-

Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. AF115509 and AF115510.

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